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Impact of traumatic brain injury on sleep structure, electrocorticographic activity and transcriptome in mice

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ABSTRACT

Traumatic brain injury (TBI), including mild TBI (mTBI), is importantly associated with vigilance and sleep complaints. Because sleep is required for learning, plasticity and recovery, we here evaluated the bidirectional relationship between mTBI and sleep with two specific objectives: (1) Test that mTBI rapidly impairs sleep–wake architecture and the dynamics of the electrophysiological marker of sleep homeostasis (i.e., non-rapid eye movement sleep delta (1–4 Hz) activity); (2) evaluate the impact of sleep loss following mTBI on the expression of plasticity markers that have been linked to sleep homeostasis and on genome-wide gene expression. A closed-head injury model was used to perform a 48 h electrocorticographic (ECoG) recording in mice submitted to mTBI or Sham surgery. mTBI was found to immediately decrease the capacity to sustain long bouts of wakefulness as well as the amplitude of the time course of ECoG delta activity during wakefulness. Significant changes in ECoG spectral activity during wakefulness, non-rapid eye movement and rapid eye movement sleep were observed mainly on the second recorded day. A second experiment was performed to measure gene expression in the cerebral cortex and hippocampus after a mTBI followed either by two consecutive days of 6 h sleep deprivation (SD) or of undisturbed behavior (quantitative PCR and next-generation sequencing). mTBI modified the expression of genes involved in immunity, inflammation and glial function (e.g., chemokines, glial markers) and SD changed that of genes linked to circadian rhythms, synaptic activity/neuronal plasticity, neuroprotection and cell death and survival. SD appeared to affect gene expression in the cerebral cortex more importantly after mTBI than Sham surgery including that of the astrocytic marker *Gfap*, which was proposed as a marker of clinical outcome after TBI. Interestingly, SD impacted the hippocampal expression of the plasticity elements *Arc* and *EfnA3* only after mTBI. Overall, our findings reveal alterations in spectral signature across all vigilance states in the first days after mTBI, and show that sleep loss post-mTBI reprograms the transcriptome in a brain area-specific manner and in a way that could be deleterious to brain recovery.

1. Introduction

Traumatic brain injury (TBI) is a public health concern that causes important short- and long-term physical, cognitive and neurobehavioral impairments (McAllister, 2011). Up to 70% of TBI survivors suffer from sleep disturbances, including insomnia, fatigue and somnolence (Cohen et al., 1992; Duclos et al., 2014; Orff et al., 2009), which are among the most common and persistent symptoms after both moderate/severe and mild TBI (mTBI) (Ayalon et al., 2007; Mahmood et al., 2004; Pillar et al., 2003). Sleep alterations have been found to be associated with several comorbidities and with impaired quality of life (Chaput et al., 2009; Fichtenberg et al., 2000; Hou et al., 2013; Ouellet et al., 2004; Schiehser et al., 2014). Importantly, these disturbances could

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interfere with brain recovery (Meerlo et al., 2009; Ouellet et al., 2004), which likely originates from the key roles of sleep in brain plasticity, learning and memory consolidation (Diekelmann and Born, 2010; Ohlmann and O'Sullivan, 2009).

Animal models are required to unravel the pathophysiology of sleep disturbances after mTBI and to understand the role of sleep in brain recovery in this context. An initial step is to describe how these disturbances develop. Despite the abundant literature on sleep–wake disturbances weeks to months following TBI of all severities in humans, less is known about sleep characteristics in the immediate days following injury, even when it comes to studies in rodents. Recent studies using a piezoelectric cage system showed that brain-injured mice had an increased percentage of rest and an increased duration of rest bouts in the first few hours or the first week after a mild or moderate TBI (Rowe et al., 2013, 2014). Another group showed a decreased ability to maintain prolonged wakefulness during the active (dark) period in the first three days after a moderate-severe TBI using EEG recording in mice, which was indexed by more bouts of wakefulness of shorter duration (Willie et al., 2012). Similar findings were reported one week post-mTBI (Lim et al., 2013). Importantly, this last study also reported alterations in the mean EEG power spectra measured during wakefulness, non-rapid eye movement sleep (NREMS) as well as rapid eye movement sleep (REMS) one week post-mTBI (Lim et al., 2013). However, to our knowledge, the progression of electroencephalographic (EEG) activity during vigilance states remains unexplored in the first days after mTBI. This includes the dynamics of EEG delta activity (1–4 Hz) during NREMS, which index a sleep homeostatic process (Borbély, 1982) thought to underlie the recovery function of sleep.

Alterations in neuronal network connectivity and the inflammatory response are among potential mechanisms responsible for sleep–wake disturbances after mTBI. Indeed, imaging and histology studies presented evidence of subtle brain injuries in mTBI such as diffuse axonal injuries (Bazarian et al., 2007; Blumbergs et al., 1995; Browne et al., 2011; Inglese et al., 2005). Also, extensive axonal and dendritic degeneration and decreased synaptic density were reported after mTBI (Gao and Chen, 2011). Given that sleep depends on the activity of neuronal networks (Krueger et al., 2008), these alterations could contribute to sleep alterations post-mTBI. In parallel, secondary injury processes are immediately activated following brain injury. Glial cells release inflammatory mediators, such as interleukin-1 (IL-1) and tumor necrosis factor alpha (TNFα) (Bachstetter et al., 2013; Helmy et al., 2011; Rowe et al., 2013). These cytokines have specifically been shown to promote sleep and to alter the sleep EEG (Krueger et al., 2011).

Changes in the expression of genes involved in neuronal plasticity and regrowth after mTBI may also directly modulate sleep structure and EEG. Neuronal growth and survival is associated with high expression of neurotrophic factors and plasticity genes (Huang and Reichardt, 2001). Research has shown that some of these elements may guide regrowth of damaged neurons in developed organisms (Deister and Schmidt, 2006). One such element is brain-derived neurotrophic factor (BDNF) that seems to profit brain recovery after TBI (McAllister et al., 2012; Rostami et al., 2011). The expression of plasticity genes such as *Bdnf, Homer1a* and *Fos* or of their protein product is increased in the hippocampus and cortex during the first hours after mTBI (Abrous et al., 1999; Colak et al., 2012; Crack et al., 2009; Hicks et al., 1999). On the other hand, the expression of several of these plasticity elements is decreased a few days after injury (Colak et al., 2012; Hou et al., 2012; Wu et al., 2010). Importantly, most of these plasticity markers were proposed to have a role in sleep regulation and were shown to respond to sleep deprivation (SD) (El Helou et al., 2013; Huber et al., 2007; Maret et al., 2007; Mongrain et al., 2010). In addition to a probable implication of changes in plasticity genes in sleep-wake disturbances post-TBI, this points to an impact of sleep loss on brain recovery after TBI.

In the present study, we used a closed-head injury model of mTBI to test the hypothesis that mTBI disturbs sleep–wake architecture and the dynamics of low frequency EEG activity in the first two days following injury. In parallel, we assessed if sleep loss is detrimental to brain gene expression following mTBI by measuring the effect of two consecutive days of enforced wakefulness post-mTBI on several plasticity markers, with focus on those linked to sleep homeostasis, and on the transcriptome in both the cerebral cortex and the hippocampus. The cerebral cortex, a direct target of the injury, was chosen because of its predominant role in the generation of delta activity (Amzica and Steriade, 2000). The hippocampus was targeted because of its important plasticity properties (Meerlo et al., 2009) and its vulnerability to TBI (Kernie and Parent, 2010). Closed-head injury was performed since it does not involve a craniotomy or a craniectomy allowing for both a more stable electrocorticographic (ECoG) recording montage and for a closer parallel to injuries observed in humans. Using this model, our results support that mTBI acutely alters the capacity to sustain wakefulness and reveal that it impacts ECoG activity in all vigilance states as well as the dynamics of slow ECoG activity during wakefulness. Moreover, our findings show that sleep loss following mTBI affects the brain transcriptome in a manner that depends on the targeted areas and that likely modulates brain recovery after injury.

2. Material and methods

2.1. Animals and protocols

Male C57BL/6 J mice purchased from Jackson Laboratories were used for two different experiments ($n = 51$). Animals were housed in individual cages in a 12 h light/12 h dark cycle at a temperature between 23 and 25 °C with food and water available *ad libitum*, and were acclimated to these conditions for at least 6 weeks before experiments. Mice were studied between 10 and 21 weeks of age, when their weight was around 30 g to reduce mortality due to TBI (see Flierl et al., 2009; Stahel et al., 2009). Mice were either subjected to a mTBI or a control Sham surgery as detailed below, which was preceded by 5 days of animal handling (5 min/day) to decrease stress due to manipulation by experimenters. All experimental procedures were approved by the Ethical Committee for Animal Experimentation of the Research Center of the Hôpital du Sacré-Coeur de Montréal in accordance with Guidelines from the Canadian Council on Animal Care.

2.1.1. Experiment 1 (ECoG measurement, Fig. 1A)

Fourteen mice (19 ± 0.5 weeks, 29.7 ± 1.0 g) were subjected to mTBI or Sham surgery ($n = 7$ per group, matched for weight) in the late afternoon (between ZT8 and ZT11 with ZT0 referring to Zeitgeber time 0, the onset of the light period, and ZT12 to the onset of the dark period). Surgeries were performed at this time, the latest possible in the light period, to ensure the shortest interval between surgery and the start of the recording, and thus to capture the earliest effect of mTBI. During that same surgical procedure, implantation of electrodes for ECoG and electromyographic (EMG) recording was performed as detailed in Section 2.3. Mice were cabled the next morning before light onset and recordings started at light onset (ZT0) for both mTBI and Sham animals, thus 13 to 16 h post-mTBI. ECoG/EMG was continuously recorded for 48 h (Day 1 and Day 2; Fig. 1A).

2.1.2. Experiment 2 (gene expression measurement, Fig. 1B)

Thirty-seven mice (14 ± 0.2 weeks, 27.2 ± 0.4 g) were used and separated into two surgical conditions (mTBI vs. Sham) and two

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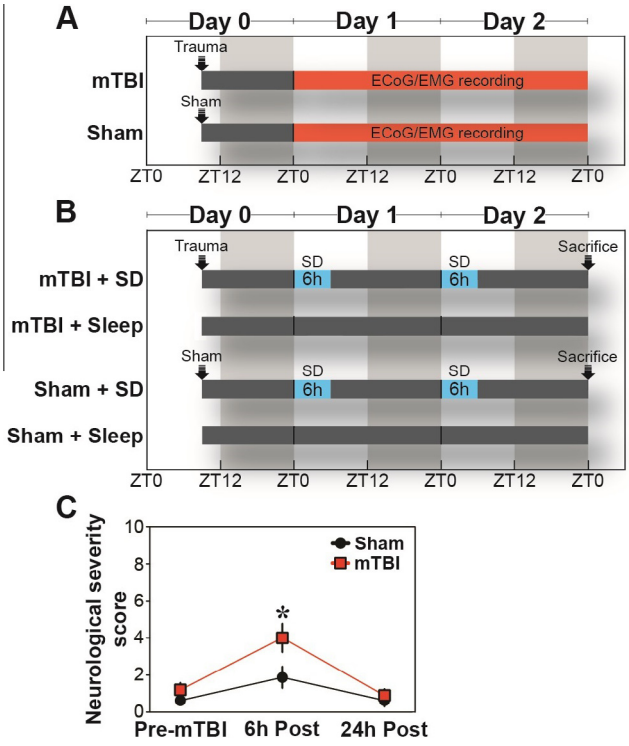

Fig. 1. (A) Schematic view of the ECoG/EMG recording protocol. mTBI was performed between ZT8 and ZT11 and was immediately followed by an electrode implantation surgery. The morning of the following day, mice were cabled around 15 min before ZT0 and ECoG/EMG were recorded continuously for 48 h starting at light onset. Sham control mice were recorded in parallel, and recordings were performed in groups of 4 to 6 mice. Gray bars indicate undisturbed/spontaneous wakefulness and sleep behavior (same in B), and red bars indicate ECoG/EMG recorded undisturbed/spontaneous wakefulness and sleep behavior. Light gray areas indicate dark periods (same in B). (B) Schematic view of the gene expression protocol. Mice were initially divided into two different groups in which animals were either submitted to mTBI or Sham surgery between ZT8 and ZT11. For each of these conditions, half of the animals were submitted to two consecutive days of 6 h sleep deprivation (SD), taking place between ZT0 and ZT6. All animals were then sacrificed on the morning of the third day for brain area sampling. (C) Neurological Severity Scale (NSS) score from an initial cohort of animals ($n = 10$ mTBI and 8 Sham). Only mTBI mice showed a significant increase in NSS score 6 h after injury in comparison to before injury ($t = 3.2$, $p < 0.01$ indicated by the star).

treatments (SD vs. Control). The first group (referred to as mTBI-SD, $n = 10$) underwent a mTBI followed by two consecutive days of 6 h SD starting at ZT0. Mice were sacrificed between ZT0 and ZT1 in the morning of the third day after 18 h of recovery following the second SD. This was performed to avoid measuring the acute effect of SD. The second group (mTBI-Control, $n = 9$) underwent the same mTBI surgery but without disrupting their sleep/wake cycle until sacrifice on the third day. The third group (Sham-SD, $n = 9$) underwent all the manipulations of the mTBI-SD group without the injury. The fourth group (Sham-Control, $n = 9$) underwent a Sham surgery and were kept under an undisturbed sleep-wake cycle until sacrifice. SD was performed by gentle handling as previously performed (Franken et al., 1991; Massart et al., 2014).

2.2. Traumatic brain injury

2.2.1. Closed-head injury

For Experiment 1 (ECoG measurement), mTBI and Sham surgeries were performed under deep ketamine/xylazine anesthesia (120/10 mg/kg, i.p. injection) to allow for subsequent electrode implantation surgery (see below). For Experiment 2 (gene expression measurement) involving sampling of brain areas, mTBI and Sham surgeries were performed under isoflurane anesthesia (4% for initiation and 2% for maintenance). mTBI was induced using a weight-drop model of closed-head injury similar to previously described (Flierl et al., 2009; Stahel et al., 2009). Briefly, anesthetized mice were shaved from the ears to the eyes and the skin was washed with 70% ethanol. A 1.5 cm longitudinal skin incision was performed and the impact point was marked (3 mm lateral right to midline and 3 mm posterior to bregma). mTBI was performed by dropping a 329 g rod from a 1-cm height. Skull fracture was observed for 7 out of 26 mice. Sham animals were used to compare trauma effects and were submitted to the exact same surgical procedures except that the weight was slowly brought to the animal's head and lifted back up (not dropped). Immediately following the impact, oxygen (2 L/min) was administered per inhalation to all mice for 5–10 min. The skin incision was then closed with sutures and the animal's behavioral state was frequently monitored.

2.2.2. Neurological Severity Scale (NSS, Fig. 1C)

A 1-cm height for weight-drop was chosen based on the ability to induce an injury considered as mild, which was evaluated using a NSS test performed on a separate cohort of animals ($n = 10$ mTBI and 8 Sham; surgeries under ketamine/xylazine anesthesia). The NSS test was a standardized 10-point scale assessing neurological function that has been used for closed-head injuries (Flierl et al., 2009; Stahel et al., 2000). It represents an abbreviated version of the original 25-points scale (Chen et al., 1996) and includes 10 parameters representative of overall motor functioning, alertness and physiological behaviors (see also El Helou et al., 2013). Fig. 1C shows that following TBI performed using a weight dropped from 1 to 1.3 cm, the NSS score was moderately but significantly increased 6 h post-surgery, and reverted to normal 24 h post-injury. Surgical procedures did not significantly increase NSS score in Sham mice. Since injuries induced from 1.3 to 1.5-cm height resulted in a 40% mortality rate in our conditions, a 1-cm height was therefore chosen for Experiments 1 and 2 to maximize survival (3.7% mortality rate, 1/27) and ensure mild injury.

2.3. ECoG/EMG electrode implantation surgery

ECoG/EMG electrode implantation was performed as detailed previously (El Helou et al., 2013; Massart et al., 2014), after undergoing either mTBI or Sham surgery. Briefly, mice were placed in a stereotaxic frame, and two gold-plated screws (1.1 mm diameter) served as ECoG electrodes and were screwed through the skull over the right cerebral hemisphere (anterior: 1.7 mm lateral to midline, 1.5 mm anterior to bregma; posterior: 1.7 mm lateral, 1.0 mm anterior to lambda). An additional gold-plated screw placed on the right hemisphere (6 mm lateral, 3 mm posterior to bregma) served as a reference. Three other screws were implanted as anchors over the left hemisphere. EMG electrodes were made out of two gold wires inserted between neck muscles. ECoG/EMG electrodes were cemented to the skull and soldered to a connector. Implantation surgeries took approximately one hour to perform and adequate measures were taken to maintain anesthesia if required (i.e., 1–2% isoflurane in case of response to toe pinch). Mice were put back in individual cages to recover from surgery and were allowed 13–16 h of recovery before cabling to a swivel contact and recording (Fig. 1A).

2.4. ECoG recording and analyses

Continuous 48 h ECoG/EMG acquisition was performed at a sampling frequency of 256 Hz using Lamont amplifiers and the software Harmonie (Natus, San Carlos, CA, USA). Wakefulness, NREMS, and REMS were visually assigned to individual 4-s epochs,

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and artifacts were simultaneously identified. For each day, the number of minutes in vigilance states per h was calculated. The number of long bouts of wakefulness (lasting at least 16-min) was calculated per 24 h, and per 12 h light and dark periods. The mean duration of individual bouts of vigilance states was computed for 24 h. The ECoG signal of artifact-free epochs was subjected to spectral analysis using fast Fourier transform to calculate ECoG power density per 0.25-Hz bin between 0.75 and 30 Hz separately for wakefulness, NREMS and REMS, and to calculate delta activity (1–4 Hz) during NREMS and wakefulness. Vigilance state spectra were averaged for 24 h and presented in relative activity calculated as a percentage of the mean of all Hz-bin of all states of the first recorded 24 h (Day 1; Fig. 1A). Delta activity was expressed as the percentage of the 24 h mean of Day 1. For NREMS, delta activity was averaged for 12 intervals during light periods, for which an equal number of epochs contributed, and 6 intervals during dark periods (Curie et al., 2013; El Helou et al., 2013). The opposite was performed for delta activity during wakefulness.

2.5. Reverse transcription and quantitative PCR

Mice were sacrificed by cervical dislocation, brains were immediately sampled and bilateral motor, somatosensory and visual cortex and bilateral whole hippocampi were rapidly frozen on dry ice. Bilateral sampling was performed to ensure sufficient biological material for measurements and to assess the combined impact of mTBI and SD on global brain function. RNA extraction, reverse transcription and quantitative PCR (qPCR) were performed similar to previously (El Helou et al., 2013; Massart et al., 2014). Briefly, RNA was extracted separately for the cerebral cortex and the hippocampus region using RNeasy Lipid Tissue Mini kit (Qiagen, Toronto, ON, Canada). RNA amount was verified with a NanoDrop ND-2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA), and the quality of RNA was assessed by agarose gel electrophoresis. Then, 500 ng of RNA was used for reverse transcription using random hexamers and SuperScript II reverse transcriptase (Invitrogen, Burlington, ON, Canada) according to standard procedures. qPCR was performed using a Viia7 real-time cycler (Life Technologies, Burlington, ON, Canada). Individual mouse cDNA were diluted and used in 10-µL reactions with TaqMan Fast advanced Master Mix reagent (Life Technologies) under standard cycling conditions: 95 °C for 20 s, followed by 40 cycles of 95 °C for 1s and 60 °C for 20 s. Each PCR reaction was done in triplicate. The three most stable endogenous controls were selected among *Actin*, TATA-box binding protein (*Tbp*), Beta-glucuronidase (*GusB*), and Ribosomal protein S9 (*Rps9*), using Expression Suite v1.0 (Life Technologies). Relative quantification was calculated using the modified ΔΔCt method from Expression Suite v1.0.

The mRNA level of selected plasticity markers (*Arc*, *Bdnf*, *Fos*, *Homer1a*), adhesion and synaptic molecules (Ephrins: *EfnA3, EfnB3*; Eph receptor: *EphA4*; and fibroblast growth factor 1 (*Fgf1*)), hypoxia-inducible factor 1-alpha (*Hif1a*) and the heat-shock protein *DnajB5* was quantified. In addition to plasticity genes linked to sleep homeostasis (i.e., *Arc*, *Bdnf*, *Fos*, *Homer1a*), Ephrins and Eph receptors seemed particularly relevant because of their role in synaptic plasticity and their altered expression after TBI (Hånell et al., 2012; Murai and Pasquale, 2011; Theus et al., 2010). The latter also applies to *Hif1a* (Higashida et al., 2011). *DnajB5* was selected because of its hypothesized role in sleep homeostasis (Mongrain et al., 2010), and because heat-shock protein pathways seem to benefit brain recovery after TBI (Eroglu et al., 2014). The list of primers, probes and TaqMan Gene expression assays used is provided in Table S1 (Supplementary material). Primers and probes were purchased from Life Technologies, Invitrogen or Operon. Other gene expression measurements were performed in an identical manner for the validation of additional targets selected from genome-wide analysis (see Section 2.6).

2.6. Next-generation sequencing (RNA-Seq)

Fifty bases paired-end sequencing was conducted at the Institut de Recherches Cliniques de Montréal (IRCM, Montreal, QC, Canada) with an Illumina Hi-Seq 2000 according to standard manufacturer instructions (Illumina, San Diego, CA, USA). This was performed using the same RNA samples of the four testing groups described above for gene expression experiment (Fig. 1B: mTBI-SD, mTBI-Control, Sham-SD and Sham-Control). Library preparation and sequencing were independently performed on three individual pools of 3 mice per group composed of 400 ng of RNA per mouse. Strand-specific libraries were prepared by IRCM according to manufacturer instructions (Illumina), and three samples were run per sequencing lane.

Base call, cleaning, genomic alignment, normalization and statistical analyses of RNA-Seq data were performed by the IRCM. Base calls were made using the Illumina CASAVA 1.8 pipeline. The quality of the reads was checked with FastQC v0.10.1 (Andrews, 2014). Low quality bases (any leading or trailing base with a quality below 3, and any window of 4 bases with an average quality below 15) were removed with Trimmomatic v0.32 (Bolger et al., 2014). The total number of sequenced paired reads for each sample was between 58 and 85 million, with average Phred quality score between 35 and 37 and % duplicate between 12.3 and 16.3. After trimming, sequences were aligned to the mouse reference genome mm9 using Tophat v2.0.10 (Kim et al., 2013). Reads aligned to annotated genes were counted with HTSeq-count v0.5.3 (Anders et al., 2014). The annotation (GTF) file used for both TopHat alignment and gene quantification was downloaded from Ensembl (release version 66). Normalization of the raw reads counts relative to sequencing depth and calculations of differential gene expression were performed with DESeq2 v1.4.5 (Love et al., 2014). A single factor design was used for computations of differential expression where samples belonging to different conditions were compared directly. Significance levels were adjusted using false discovery rate (FDR) (Benjamini and Hochberg, 1995). Venn diagrams of differentially expressed genes were built with Venny (Oliveros, 2007) and heatmap representations with Gene-E (Broad Institute, Cambridge, MA, USA). Biological function analyses of differentially expressed transcripts were performed using Ingenuity Pathway Analysis (IPA, www.ingenuity.com; Qiagen), which assess the significance of the association between ensembles of transcripts and gene networks linked to biological functions using right-tailed Fisher Exact tests.

2.7. Statistical analyses

The effect of mTBI on the time course of vigilance states and of delta activity was assessed using repeated-measure two-way analyses of variance (ANOVA) with factors Condition (mTBI vs. Sham) and Hour or Interval. Significance levels were adjusted for repeated measures using Huynh–Feldt correction when appropriate. The impact of mTBI on the mean duration of bouts of vigilance states and on the number of long wakefulness bouts per 24 h or 12 h was investigated by *t*-tests. ECoG spectra of the different vigilance states of mice submitted to mTBI and Sham surgery were also compared using *t*-tests (analysis and graphical representation performed for Hz-bins between 0.75 and 25 Hz). Changes in gene expression after mTBI and SD were investigated by two-way ANOVA with factors Condition (mTBI vs. Sham) and Treatment (SD vs. Control). Statistical analyses were performed using Statistica (Statsoft, Tulsa, OK, USA). Threshold for statistical significance was set to 0.05 and results are reported as mean ± SEM.

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3. Results

3.1. Decreased long wake bouts after mTBI

The overall time course of vigilance states showed the expected dynamics over the light–dark cycle in both mTBI and Sham mice with sleep prevailing over wakefulness during the light period, while wakefulness was predominant over NREMS and REMS during the dark (active) period. The time course of wakefulness, NREMS and REMS did not significantly differ between mTBI and Sham mice for both Day 1 and Day 2 (Fig. 2A). Also for both days, the total duration (Fig. 2A) and the 24 h mean duration of individual bouts (Table S2) of wakefulness, NREMS and REMS did not significantly differ between mTBI and Sham mice. Given that previous literature specifically suggests that mTBI mostly impacts the capacity to sustain wakefulness (Lim et al., 2013) and wakefulness quality, as measured by EEG in humans (Gosselin et al., 2009), we evaluated whether mTBI mice showed modifications in the number of long bouts of wakefulness. For Day 1, mTBI mice showed significantly less bouts of 16 min of wakefulness than Sham mice both for the 24 h and the 12 h dark period (Fig. 2B). This difference was no longer present on Day 2, during which the number of long wakefulness bouts in mTBI mice returned to the level observed in Sham mice.

3.2. Modified ECoG spectra after mTBI

For Day 1, power spectra during wakefulness and REMS did not significantly differ between mTBI and Sham mice (Fig. 3A, top and bottom left panels). However, the activity in frequencies between 4.75 and 5.5 Hz during NREMS was significantly higher in mTBI mice than in Sham mice (Fig. 3A, middle left panel). The portrait was different on Day 2, where mTBI mice expressed higher spectral activity than Sham mice in many theta (4–8 Hz) and some beta (16–25 Hz) frequencies during all three vigilance states (Fig. 3A, right panels). More precisely, during wakefulness, activity between 3.75 and 5.75 Hz and that of two faster Hz-bins was higher in mTBI than in Sham mice. During NREMS, activity between 3.5 and 6.75 Hz and in most Hz-bins between 14.75 and 25 was higher in mTBI than in Sham mice, while during REMS, the group differences concerned the activity between 3.25 and 5 Hz and two Hz-bins between 21.75 and 23 Hz. Overall, this indicated that on the second day after mTBI, injured mice expressed more activity than Sham mice in both slow (i.e., high delta/low theta) and fast (i.e., beta) frequencies during all vigilance states.

3.3. Altered wake delta dynamics after mTBI

To investigate if mTBI impacts the sleep recovery process (i.e., sleep homeostasis), the 48 h time course of NREMS delta activity was compared between mTBI and Sham mice. In addition, because of our specific observations of alterations in wakefulness after mTBI (i.e., decreased number of long bouts, increased slow ECoG activity), the effect of mTBI on the time course of delta activity during wakefulness was also investigated. During wakefulness, the 48 h time course of delta activity was significantly affected by mTBI (Fig. 3B, upper panel). During Day 1, a light–dark difference in delta activity observed in Sham mice (e.g., higher wake delta in light than in dark) was absent in mTBI mice resulting in higher activity in mTBI than in Sham mice for several intervals during the dark period. The light–dark difference seemed restored in mTBI mice on Day 2 but the daily rhythm was expressed at higher delta activity levels than in Sham mice, which was associated with higher delta in mTBI than Sham mice for several intervals during both the light and dark periods. Thus, the time course of delta activity during wakefulness seemed similar on Day 1 and Day 2 in Sham mice whereas for mTBI mice, it was importantly blunted on Day 1 and at a higher level than Sham mice on Day 2. In contrast, during NREMS, the time course of delta activity was not significantly affected by mTBI (Fig. 3B, lower panels). In general, the daily dynamics of NREMS delta activity was blunted on Day 1 compared to Day 2 for both mTBI and Sham mice. Because of this observation in Sham, this may result from a residual effect of the ECoG/EMG implantation surgery, which included anesthesia that

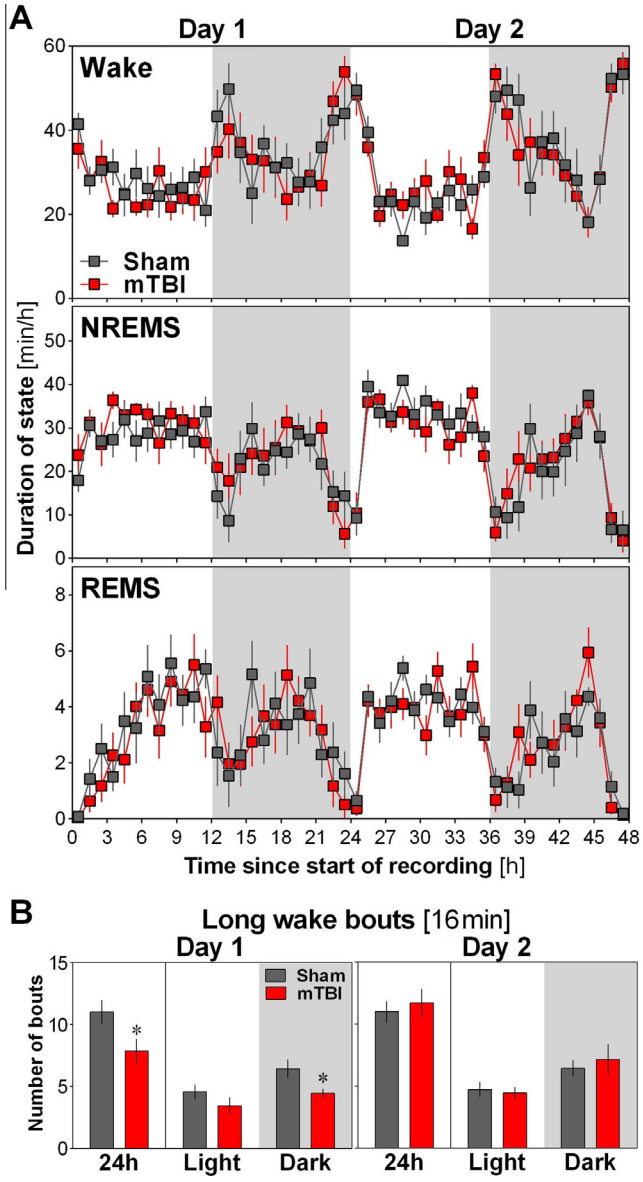

Fig. 2. (A) Forty-eight-hour time courses of vigilance state duration in mTBI and Sham mice measured using ECoG/EMG recording. The upper panel shows the time course of wakefulness, the middle panel that of NREMS and the lower panel that of REMS. The time course of the three vigilance states did not significantly differ between mTBI and Sham mice on both Day 1 (interaction: wakefulness $F_{23,276} = 0.7$, $p = 0.9$; NREMS $F_{23,276} = 0.7$, $p = 0.9$; REMS $F_{23,276} = 0.8$, $p = 0.8$) and Day 2 (interaction: wakefulness $F_{23,276} = 0.7$, $p = 0.8$; NREMS $F_{23,276} = 0.7$, $p = 0.8$; REMS $F_{23,276} = 1.0$, $p = 0.5$). The total duration of vigilance states was also similar between mTBI and Sham mice on Day 1 and 2 (Condition effect wakefulness: $F_{1,12} < 1.3$, $p > 0.2$; NREMS: $F_{1,12} < 1.9$, $p > 0.2$; REMS: $F_{1,12} < 0.7$, $p > 0.4$). Gray areas indicate dark periods (same in B). (B) Number of long bouts of wakefulness (16 min) in mTBI and Sham mice averaged for 24 h, 12 h light, and 12 h dark periods separately for the first and the second recorded days. A significant Condition effect (mTBI vs. Sham) was found for 24 h and 12 h dark period only for the first recorded day ($t > 2.3$, $p < 0.05$ indicated by stars; Day 1 12 h light period: $t = 1.3$, $p = 0.2$; Day 2: $t < 0.8$, $p > 0.4$).

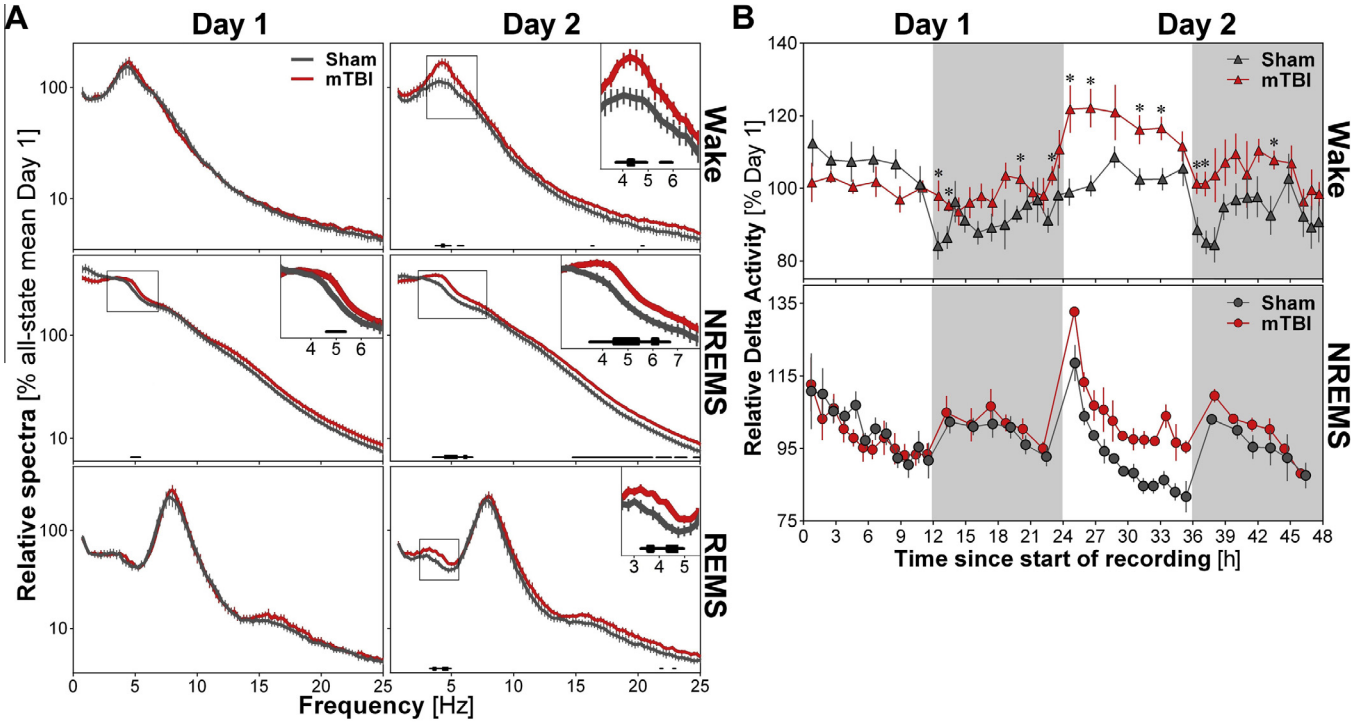

Fig. 3. (A) Wakefulness (upper panels), NREMS (middle panels) and REMS (bottom panels) relative spectral power averaged for 24 h per 0.25 Hz-bin between 0.75 to 25 Hz separately for Day 1 and Day 2 in mTBI and Sham mice. Significant group differences were observed only in NREMS for Day 1 for three bins around 5 Hz ($t > 2.2$, $p < 0.05$). For Day 2, significant group effects ($t > 2.2$, $p < 0.05$) were observed for wakefulness for Hz-bins around 5 Hz and at 16.25 and 20.25 Hz, for NREMS between 3.5 and 6.75 Hz and between 14.75 and 25 Hz, and for REMS between 3.25 and 5 Hz and between 21.75 and 23 Hz. Significant differences between mTBI and Sham mice are represented by black bars above the *x*-axis (thin bars: $p < 0.05$; thick bars: $p < 0.01$). Inserts show magnifications of indicated spectra with significant differences. (B) Forty-eight-hour time courses of relative delta activity during wakefulness (upper panel) and NREMS (bottom panel) in mTBI and Sham mice. A Condition (mTBI vs. Sham) by Interval interaction was found for relative delta activity during wakefulness ($F_{35,420} = 1.8$, $p = 0.02$). Stars indicate significant differences between mTBI and Sham mice for indicated points ($p < 0.05$, simple effect analysis). No significant Condition by Interval interaction or Condition effect was found for relative delta activity during NREMS (interaction: $F_{35,420} = 1.0$, $p = 0.5$; condition: $F_{1,12} = 1.8$, $p = 0.2$). Gray areas indicate dark periods.

was shown to modulate NREMS delta time course after SD (Nelson et al., 2010).

3.4. Expression of target genes after mTBI and SD

The mRNA expression of 10 target genes associated with plasticity and with sleep regulation or brain injury was measured in the cerebral cortex and the hippocampus after two consecutive days of 6 h SD to investigate the impact of sleep loss on plasticity markers after mTBI. In the cerebral cortex, mTBI itself did not significantly affect the expression of the 10 target genes (i.e., *Arc*, *Bdnf*, *DnajB5*, *Fos*, *Homer1a*, *Hif1a*, *EfnA3*, *EfnB3*, *EphA4*, *Fgf1*; Fig. 4). However, two days of SD importantly affected the expression of many of these genes. More precisely, SD decreased the expression of *Arc*, *Bdnf*, *Dnajb5*, *Fos*, *Homer1a* and *EphA4*. This is in striking contrast with the acute effect of SD (i.e., gene expression measured immediately at the end of the 6 h SD), which increases the expression of most of these genes (Maret et al., 2007; Massart et al., 2014; Mongrain et al., 2010). SD also increased the expression of *EfnA3* in the mouse cerebral cortex. These SD-dependent changes were observed for both mTBI and Sham mice as no significant interaction between mTBI and SD was observed. In contrast, in the hippocampus, significant interactions between Condition (mTBI vs. Sham) and Treatment (SD vs. Control) were found for *Arc* and *EfnA3*, with SD decreasing their mRNA level only in mTBI mice (Fig. 4). An effect of SD was only observed for *EfnB3* in the hippocampus showing that SD decreased *EfnB3* mRNA in both mTBI and Sham mice. Again, no significant main effect of mTBI was observed.

3.5. Changed transcriptome after mTBI and SD

To facilitate identification of pathways affected by mTBI and sleep loss, we investigated the genome-wide effect of mTBI and SD in the cerebral cortex and hippocampus by performing RNA-Seq using samples from Experiment 2 (Fig. 1B). Consistent with our mild model of brain injury, when comparing animals that were not submitted to SD (mTBI-Control vs. Sham-Control), few genes were affected by mTBI. For the cerebral cortex, none reached a genome-wide level of statistical significance (i.e., FDR < 0.05). With a threshold of $p < 0.01$, 17 transcripts were affected by mTBI in the cerebral cortex and 60 transcripts in the hippocampus (Tables S3 and S4, Supplementary material). Biological function analysis revealed that these transcripts were significantly enriched in genes involved in inflammatory response (e.g., *S100a8*, *Lgal3*, *Cxcl*), immunological disease (e.g., *S100a9*, *Lgals3*, *C4a*/*C4b*), cell-to-cell signaling and interaction (e.g., *Gfap*, *B2m*, *Cxcl6*), and immune cell trafficking (e.g., *Anxa2*, *Lgals3*, *Cd9*) in both brain areas (Table S5 and S6).

Interestingly, SD seemed to differentially affect the transcriptome in mTBI and Sham mice in a brain region-specific manner. In the cerebral cortex, 172 transcripts were significantly changed by SD (FDR < 0.05) in mTBI mice while 100 transcripts were significantly modified by SD in Sham mice (Table S5 and S6). Only 44 of these were common between mTBI and Sham mice (Fig. 5A, upper panel). Mice submitted to mTBI had more transcripts responding to SD by both an increase and a decrease in comparison to Sham mice (Fig. 5A, lower panel). Transcripts changed in both mTBI and Sham mice were significantly enriched in pathways associated with

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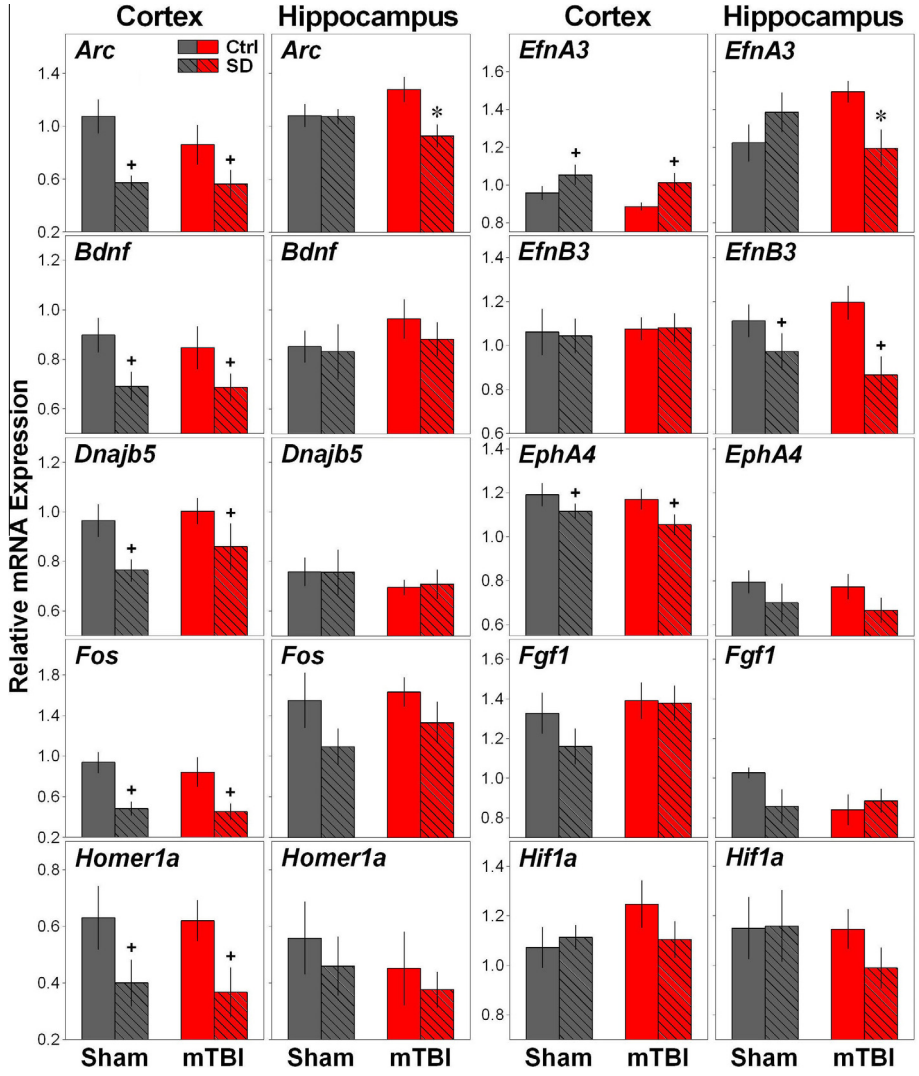

Fig. 4. Effect of mTBI and two consecutive days of 6 h sleep deprivation (SD) on the expression of plasticity and other target genes measured by qPCR on the third morning after surgery in the cerebral cortex and hippocampus. In the cerebral cortex, no significant interaction between Condition (mTBI vs. Sham) and Treatment (SD vs. Control (Ctrl)) and no significant Condition effect was found. Significant Treatment (SD vs. Control) effect was found for *Arc*, *Bdnf*, *DnajB5*, *Fos*, *Homer1a*, *EfnA3* and *EphA4* ($F_{1,33} > 4.3$, $p < 0.05$, indicated by +). In the hippocampus, a significant Condition by Treatment interaction was found for *Arc* and *EfnA3* ($F_{1,33} > 4.6$, $p < 0.04$, indicated by stars), and a tendency for Condition by Treatment interaction was observed for *Fgf1* ($F_{1,33} = 2.6$, $p = 0.1$). No significant Condition effect was found, but a significant Treatment (SD vs. Control) effect was found for *EfnB3* ($F_{1,33} = 8.6$, $p < 0.01$ indicated by +), and a tendency for a significant Treatment effect was observed for *Fos* ($F_{1,33} = 3.4$, $p = 0.07$).

circadian rhythm signaling (i.e., *Arntl*, *Nr1d1*), role of NFAT in immune response regulation (e.g., *Csnk1g2*, *Mapk3*), and corticotropin releasing hormone signaling (i.e., *Mapk3*, *Mef2c*) (Table S5). Transcripts significantly changed by SD **only in mTBI mice** were associated with GABA receptor signaling (e.g., *Adcy8*, *Gabrd*), Ephrin A signaling (i.e., *EfnA3*, *EphA10*), serotonin receptor signaling (i.e., *Adcy8*, *Maoa*), cell death and survival (e.g., *Fkbp8*, *Hif1a*), and cellular development (e.g., *Faim2*, *Gfap*, *Dbn1*) (Table S5). In contrast, transcripts changed by SD **only in Sham mice** were significantly associated with the antiproliferative role of somatostatin receptor 2 (e.g., *Cdkn1a*, *Gng4*), GADD45 signaling (i.e., *Ccne2*, *Cdkn1a*), PI3/AKT signaling (e.j., *Ptgs2*, *Synj2*), IL-8 signaling (e.g., *Gng4*, *Gng12*), cell death and survival (e.g., *Enc1*, *Nr4a2*), and cell-mediated immune response (e.g., *Il17ra*, *Il4r*, *Egr3*) (Table S5).

In the hippocampus, mTBI mice showed much less transcripts differentially affected by SD compared to Sham mice (11 vs. 48; Fig. 5B upper panel), with only two transcripts being common between mTBI and Sham groups (Tables S9 and S10). This was again observed for both increases and decreases after SD (Fig. 5B, lower panel). Unexpectedly, the two transcripts changed by SD in both mTBI and Sham mice were *Dbp* and *Nr1d1*, two known clock genes giving rise to transcriptional regulators, which again emphasize a major responsiveness of the molecular clock system to the 2-day SD paradigm used here. Transcripts significantly changed by SD **only in mTBI mice** were associated with inflammatory response, cellular function and maintenance, behavior and cell-mediated immune response for which *Bhlhe41* represented a major contributor (Table S6). Of note is that BHLHE41 (basic helix-loop-helix family member e41), also known as DEC2, is a transcription factor involved in circadian rhythm regulation that has been shown to have modulatory effects on both sleep duration and the susceptibility to sleep loss (He et al., 2009; Pellegrino et al., 2014). We also observed *Bhlhe41* expression to be significantly increased by SD in the cerebral cortex of both mTBI and Sham mice (Table S5, Fig. S1). In contrast, transcripts changed by SD **only in Sham mice** were significantly associated with axon guidance signaling (e.g., *Sema6b*, *Tuba1a*), EIF2 signaling (e.g., *Rpl13*, *Rps25*), inflammatory response (e.g., *Tubb2a*, *Mif*), cell death and survival

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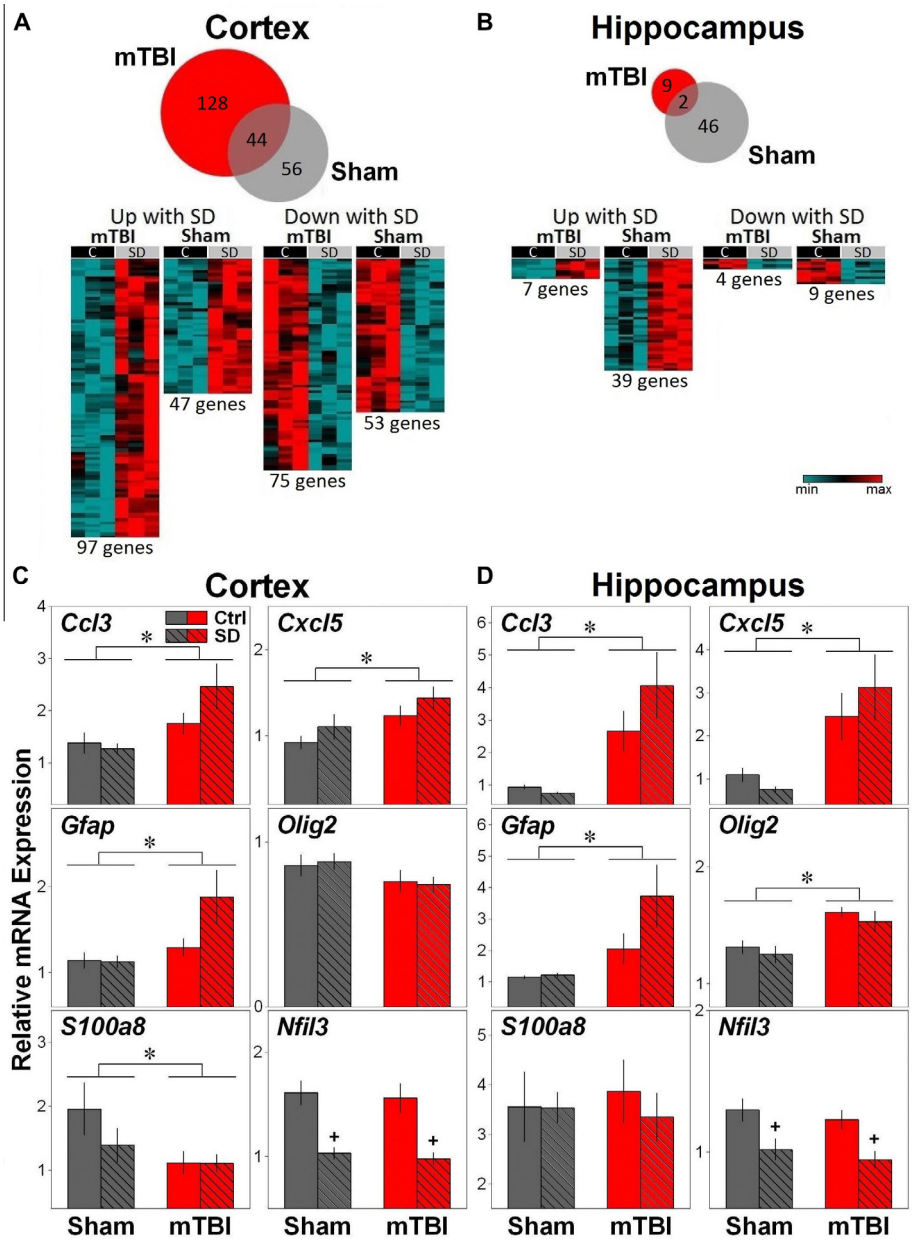

Fig. 5. Venn diagrams (upper panels) and heatmap representations (lower panels) of genes differentially expressed in mTBI and Sham mice after two days of 6 h sleep deprivation (SD) in the cerebral cortex (A) and hippocampus (B) as measured using high-throughput sequencing (RNA-Seq). Venn diagrams show the overlap between genes differentially expressed (FDR < 0.05) in mTBI and Sham mice. In the cerebral cortex, 25.6% of genes differentially expressed after SD in mTBI mice overlap with those differentially expressed in Sham mice, whereas 44% of those in Sham mice overlap with those of mTBI mice. For the hippocampus, 18.2% of genes differentially expressed after SD in mTBI mice overlap with those differentially expressed in Sham mice, while only 4.2% of those in Sham mice overlap with those of mTBI mice. Heatmaps represent the expression of the same transcripts with significant differential expression after SD in mTBI and Sham mice, separately sorted according to the direction of the change with SD. For each condition (mTBI and Sham), columns refer to three pools of RNA of three control (C) and three SD mice (total nine per group). Transcripts were ordered by hierarchical clustering (complete linkage). (C and D) qPCR validations of the effect of mTBI and SD on the expression of selected transcripts on the third morning after surgery in the cerebral cortex and hippocampus. (C) In the cerebral cortex, significant Condition effects (mTBI vs. Sham, indicated by stars) were observed for *Ccl3*, *Cxcl5*, *Gfap* and *S100a8* ($F_{1,33} > 4.3$, $p < 0.05$). A significant Treatment effect (SD vs. Control (Ctrl), indicated by +) was observed for *Nfil3* ($F_{1,33} > 34.6$, $p < 0.001$). A trend for Condition by Treatment interaction was found for *Gfap* ($F_{1,33} = 2.6$, $p = 0.1$). (D) In the hippocampus, significant Condition effects (mTBI vs. Sham, indicated by stars) were observed for *Ccl3*, *Cxcl5*, *Olig2* and *Gfap* ($F_{1,33} > 8.2$, $p < 0.05$). A significant Treatment effect (SD vs. Control, indicated by +) was observed for of *Nfil3* ($F_{1,33} = 15.1$, $p < 0.001$). No Condition by Treatment interaction was found for qPCR validation in the hippocampus.

(e.g., *Gapdh*, *Npy*), and molecular transport (e.g., *Atp5d*, *Kcnj4*) (Table S6).

3.6. Enhanced expression of chemokine and glial genes after mTBI

Changes in the expression of selected transcripts were validated by qPCR. Specifically, gene expression changes were validated for two chemokines (chemokine ligand-3 (*Ccl3*; also known as macrophage inflammatory protein (MIP)-1α) and chemokine C-X-C motif ligand 5 (*Cxcl5*)) shown to respond to brain insult (Mirabelli-Badenier et al., 2011). *Ccl3* was also observed to be upregulated after mTBI (Kumar et al., 2013; Redell et al., 2013). The expression of three markers of glial activation was also measured: oligodendrocyte lineage transcription factor 2 (*Olig2*), which is increased after brain insults (Buffo et al., 2005); glia-fibrillary acidic protein (*Gfap*), a proposed biomarker of TBI (Yokobori et al., 2013); and

S100 calcium binding protein A8 (*S100a8* or macrophage-inhibiting factor related protein-8 (*Mrp8*)), a microglial marker shown to be increased after both ischemia and TBI (Engel et al., 2000; Postler et al., 1997). Finally, nuclear factor interleukin 3 regulated (*Nfil3* or *E4bp4*) was targeted because of its role in the regulation of circadian rhythms (Mitsui et al., 2001) and in neuronal survival (Junghans et al., 2004).

In the cerebral cortex, mTBI increased the mRNA expression of *Ccl3*, *Cxcl5* and *Gfap*, and decreased the expression of *S100a8* (Fig. 5C). In addition, SD decreased the mRNA level of *Nfil3*. No significant Condition (mTBI vs. Sham) by Treatment (SD vs. Control) interaction was found indicating that mTBI and SD independently changed the expression of these genes in the cerebral cortex. However, the expression of the astrocytic marker *Gfap* showed a tendency for a Condition by Treatment interaction indicating that SD tended to amplify the increase produced by mTBI. In the hippocampus, mTBI also increased the expression of the chemokines *Ccl3* and *Cxcl5* as well as the expression of the glial markers *Olig2* and *Gfap* (Fig. 5D). Again, SD only affected the expression of *Nfil3*, showing a decrease after SD compared to Control. Even if the mTBI-dependent changes in expression of *Cxcl5* and *Gfap*, in particular, seemed affected by SD, no significant Condition by Treatment interaction was observed for the hippocampus indicating that mTBI and SD mostly independently affect the expression of these selected genes.

4. Discussion

Using a murine ecological model of mild brain injury, we showed that mTBI acutely affects both the capacity to sustain long episodes of wakefulness as well as the daily dynamics of slow ECoG activity during wakefulness. In addition, we found that the activity of the cerebral cortex in the first days after mTBI is altered for several frequencies (high delta, theta, beta) in the three vigilance states. Moreover, using a genome-wide approach, we observed, 2.5 days after injury, modifications in the expression of mediators of inflammation and of glial cell function that showed some level of specificity for the cerebral cortex and the hippocampus, and which could represent molecular correlates of behavioral/electrophysiological changes. Finally, we report that two consecutive days of enforced wakefulness importantly affected gene expression after mTBI, particularly of genes involved in neuronal plasticity, synaptic function and circadian rhythm regulation, revealing pathways by which insufficient sleep can modulate recovery after TBI.

Our observation of subtle differences in sleep-wake architecture after mTBI is consistent with the mild nature of the injury for which several clinical studies have reported few changes in objective measures of wakefulness and sleep duration in days to years after injury (Gosselin et al., 2009; Khoury et al., 2013; Rao et al., 2011) despite important subjective complaints (Chaput et al., 2009; Gosselin et al., 2009; Khoury et al., 2013). We observed a specific decrease in the number of long wakefulness bouts after mTBI, indicative of a decreased capacity to maintain wakefulness, which is also consistent with previous rodent literature (Lim et al., 2013; Rowe et al., 2013, 2014; Willie et al., 2012). This difference was only found in the first recorded 24 h, which is reminiscent of a study showing that sleep measured via piezoelectric cages predominates in the first hours after TBI (Rowe et al., 2013). Interestingly, it seems that differences in ECoG spectral activity after mTBI mainly emerged when the number of long wakefulness bouts was restored to the level observed in Sham mice (i.e., on Day 2). This suggests that sleep-wake modifications are submitted to biphasic modifications in the first days after mTBI, with impairments in sustaining long wakefulness episodes occurring first, and being followed by alterations in ECoG activity captured by spectral analysis. Altered power spectra in the three vigilance states were also reported a week after mTBI in rodents (Lim et al., 2013). However, the former study found that TBI decreased activity in frequencies above 6 Hz, which is different from our observation of elevated ECoG spectral activity two days after mTBI and from increased delta during wakefulness months following mTBI in humans (Gosselin et al., 2009). This difference could be due to a different injury type (open skull vs. closed-head). In our study, larger differences between mTBI and Sham on the first recorded day after injury may have been masked by recovery from surgery. Due to our methodological conditions of closed-head injury and standard ECoG recording montage (gold standard in sleep research and spectral analysis; Franken et al., 2001), it was not possible to perform mTBI on animals previously implanted with the electrode montage. Accordingly, recovery in Sham might have prevented the observation of larger group differences on Day 1.

Of relevance, we observed a blunted dynamics of delta activity during wakefulness after mTBI, and this was specifically present on the first recorded day. Indeed, mTBI mice showed an absence of time of day-dependent changes in delta during wakefulness whereas Sham animals expressed higher wake delta during the rest (light) compared to the active (dark) period. Delta oscillations during wakefulness are generally associated with quiet wakefulness (as opposed to active wakefulness) and lapses in attention (Leemburg et al., 2010; Vyazovskiy et al., 2011), and have even been proposed as local sleep phenomena (Krueger et al., 2008; Vyazovskiy et al., 2011). Therefore, lower waking delta during the active period might ensure maximal vigilance quality. The fact that mTBI mice specifically showed higher relative waking delta during the active period further supports a specific impairment in wakefulness quality after mTBI and importantly refines the observation of impaired capacity to sustain long bouts of wakefulness. This observation might also suggest an acute impact on the circadian timing system, which is in line with findings of altered rest-activity rhythm in the first days after moderate to severe TBI in humans (Duclos et al., 2013). Alternatively, these changes may be linked to alterations in other neuronal circuits involved in sleep-wake regulation, such as hypothalamic orexin neurons or the thalamic reticular nucleus that were both shown to be modified by TBI (Baumann et al., 2009; Hazra et al., 2014; Willie et al., 2012). Of course, understanding how the initial post-injury progression of brain activity during sleep and wakefulness is associated with the progression of cognitive functions, such as the ability to adjust to new environments after mTBI, will require further studies.

The observation of a general increase in delta activity across all 3 vigilance states after mTBI suggests that this altered ECoG signature is not linked to sleep homeostasis. In addition, we found that the time course of delta activity during NREMS was mostly preserved after mTBI. Though consistent with the unaltered nighttime decay dynamics of NREMS delta activity observed months after concussion in athletes (Gosselin et al., 2009), this result should be interpreted with caution since group differences could have been hidden by interindividual variability, which is relatively important after mTBI in a closed-head configuration as well as in clinical populations. Thus, replication of these findings with more animals should be planned. In addition, measurement of the NREMS delta response to SD will be required to definitely rule out an alteration in the recovery sleep process after mTBI. This absence of changes in NREMS delta dynamics observed in combination with an altered delta dynamics during wakefulness suggests that neuronal activity during wakefulness is specifically influenced by the robust changes in markers of inflammation and glial cell function after mTBI, which we revealed by both biological pathway analysis of RNA-Seq data and qPCR validations. Indeed, we

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reported for the first time that mTBI enhanced the expression of two chemokines (*Ccl3* and *Cxcl5*) in both the cerebral cortex and hippocampus 2.5 days after injury, which supplements observations of their upregulation after brain ischemia or TBI (Kumar et al., 2013; Mirabelli-Badenier et al., 2011; Redell et al., 2013). Consistent with an induction of immune/inflammation pathways after mTBI, chemokines can be produced by microglia (Ramesh et al., 2013), the main immune cells of the central nervous system.

We also observed changes in the expression of two recognized markers of microglia activation after mTBI but specifically in the cerebral cortex: *Lgals3* (lectin galactoside-binding soluble 3 or Galactin-3) and *S100a8*. Like *Ccl3* and *Cxcl5*, *Lgals3*, which has a role in inflammation and neurodegenerative diseases (Lerman et al., 2012), was increased by mTBI in the cortex (Table S5), whereas *S100a8* was decreased. This last observation, although opposite to findings of induction in *S100a8* after ischemia and TBI (Engel et al., 2000; Postler et al., 1997), could suggest upregulation of neuroprotective pathways after brain injury since the absence of *S100a8* has been linked to neuroprotection (Ziegler et al., 2009). Microglial activation was observed after moderate TBI both in the cerebral cortex and hippocampus (Kumar et al., 2013), and our observations support that the extent and/or the properties of this activation in the two brain areas depends on injury severity. Although cytokines were shown to modulate sleep and EEG activity (Krueger et al., 2008, 2011), further studies will need to investigate how the specific markers mentioned above could contribute to changes in wakefulness and sleep structure as well as EEG.

Following mTBI, changes in the expression of markers of the brain's two other glial cell types also reveal potential sleep-modulatory mechanisms and brain area-specific effects. Astrocytes, in particular, were shown to release gliotransmitters directly affecting sleep-wake duration and EEG activity in slow frequencies during sleep (Halassa et al., 2009). We observed an important increased in the astrocytic marker *Gfap* after mTBI in both the cerebral cortex and the hippocampus, which is in line with the increased cortical level of GFAP protein observed one week after brain injury in mice (Hazra et al., 2014). This thus supports a contribution of astrocyte activation after mTBI to sleep-wake modifications in our conditions, and possibly to cognitive deficits via their modified state in the hippocampus. Interestingly, to our knowledge, we are first to report *Olig2* to be specifically increased after mTBI in the hippocampus. OLIG2 is predominantly found in oligodendrocytes and has been shown to be expressed in response to different types of brain insults (Buffo et al., 2005). Given that oligodendrocytes are importantly involved in axon myelinisation, this observation may suggest enhanced need for re-myelinisation after injury in the hippocampus. Of note is that the oligodendrocyte transcriptome was recently shown to importantly vary with the duration of wakefulness and sleep in the rodent brain (Bellesi et al., 2013), suggesting a potential involvement of sleep and wakefulness alternations in central nervous system myelinisation. Overall, our findings indicate that even a mild brain injury seems to activate the three types of glial cells in the central nervous system and this in a brain-region specific manner.

Importantly, the increase in the expression of *Gfap* after mTBI, whose protein product has been proposed as a biomarker of TBI in humans (Yokobori et al., 2013), was particularly elevated after SD in the cerebral cortex. This suggests that SD could potentiate astrocyte activation after mTBI. Even though astrocytes can contribute to neuroprotection, their activation can also mediate abundant astrogliosis with a negative impact on brain function, and blood GFAP levels were shown to predict death after severe TBI in humans (Vos et al., 2004). Our findings may thus suggest that SD could impair brain recovery after mTBI by inducing exaggerated astrocyte reactivity. In line with this, we found that mTBI mice expressed more changes in the cerebral cortex transcriptome after SD than Sham mice. Furthermore, an important effect of two days of SD on the cortical expression of genes involved in plasticity, neuroprotection and circadian rhythms was observed, which strongly suggests a modulatory impact of SD on brain function after injury. Indeed, genes generally positively associated with learning and synaptic function (e.g., *Arc*, *Bdnf*, *Fos*, *Homer1a*) were importantly downregulated after sleep loss. Downregulated expression of *Homer1a*, in particular, may impair neuroprotective pathways because the expression of *Homer1a* was suggested to have a neuroprotective role during SD (Maret et al., 2007) and a protective role in TBI via downregulating group 1 metabotropic glutamate receptors (Luo et al., 2014). In addition, because BDNF is generally associated with neuronal survival (Acheson et al., 1995), its decreased expression after SD in the cerebral cortex may impair neuronal recovery after mTBI. Finally, a major impact of the two SD days was observed on the expression of many genes involved in circadian rhythm regulation (i.e., *Arntl*, *Dbp*, *Npas2*, *Bhlhe41*, *Nr1d1*, *Per3*, *Nfil3*), which is evocative of the finding that the expression of many of these clock genes is modulated by sleep restriction in human blood cells (Möller-Levet et al., 2013). Besides their implication in circadian regulation, these genes are also linked to neuronal and synaptic plasticity (Mongrain and Franken, 2013), therefore highlighting their capacity to affect brain recovery after injury. Accordingly, our findings support that, especially in the cerebral cortex, sleep loss may reprogram the transcriptome in a manner that could be deleterious to brain recovery after mTBI.

Alternatively, SD-dependent increase in *EfnA3* and decrease in *EphA4* expression in the cerebral cortex may suggest that sleep loss is not only detrimental to brain recovery after mTBI and could rather produce some positive effects. In fact, the lack of EphA4 was shown to be positively associated with neuronal reconstruction after spinal cord injury (Goldshmit et al., 2004), likely because EphA4 can mediate spine retraction (Murai and Pasquale, 2011). Importantly, EphA4 and EfnA3 have both been implicated in synaptic plasticity and neuron-glia interactions (Murai and Pasquale, 2011), emphasizing the relevance of changes in these elements after TBI. In the hippocampus, our observation that the expression of the plasticity elements *Arc* and *EfnA3* are restored to Sham levels in mTBI mice that were submitted to SD may also suggest a potential benefit of SD after injury. Moreover, SD affected fewer genes in the hippocampus of mTBI mice than in that of Sham mice. Nonetheless, the upregulation of *Arc* and *EfnA3* after injury might also be required to stimulate neuronal growth and plasticity to recover from injury, and the ablation of these changes by SD might have adverse effects. Of interest is also our observation that biological pathways linked to both Ephrin A signaling and serotonin receptor signaling are modified by SD in the cortex only in mTBI mice, further suggesting specific modifications of plasticity and neurotransmission mechanisms after mTBI. Of course, the precise impact of these modifications on neuronal reconstruction and functional impact after mTBI will need to be addressed in future studies. This will also be required in order to reconcile findings from one study indicating that SD benefits the recovery of neurological function after TBI (Martinez-Vargas et al., 2012), and that of another study having shown that SD has neither an effect on NSS score nor on novel object recognition post-TBI (Rowe et al., 2014).

In conclusion, we confirmed a specific deficit in the capacity to sustain wakefulness in the initial hours following brain injury, and we uncover that this is associated with alterations in the dynamics of slow cortical activity during wakefulness. We also reveal important changes in cerebral cortex activity across all vigilance states in the first days after mild brain injury and a preservation of the time course of NREMS delta activity. Importantly, our findings identified novel elements that could contribute to sleep-wake and ECoG modifications, elements supporting an implication of pathways linked to immune and glial functions. Moreover, our results sug-

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gest a brain area-specific impact of mTBI on all three types of glial cells in the central nervous system. Assessment of inter-hemispheric differences in these observations, shown to be important for several plasticity genes after TBI (Hicks et al., 1999), and of their associations with changes in neuronal morphology (e.g., dendrite and spine number and shape) will help to understand the contribution of the identified elements to wakefulness and sleep phenotypes. Future studies should also consider the effect of time-of-day at which the injury is performed on brain gene expression since it was shown to impact on mortality rate (Martinez-Vargas et al., 2006). Finally, our data also expose several molecular routes by which sleep loss could crucially impact on brain recovery after a traumatic injury.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbi.2014.12.023.

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